

REMARKS

This Preliminary Amendment is made prior to examination of this application on the merits. Consideration of the application is respectfully requested in view of the above amendments and the following remarks.

Applicants earnestly believe that they are entitled to a letters patent, and respectfully solicit the Examiner to expedite prosecution of this patent application to issuance. Should the Examiner have any questions, the Examiner is encouraged to telephone the undersigned.

Respectfully submitted,

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By:

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CHIMERIC PROTEIN CONTAINING AN INTRAMOLECULAR CHAPERONE-
LIKE SEQUENCE AND ITS APPLICATION TO INSULIN PRODUCTION

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1. BACKGROUND OF THE INVENTION

1.1. TECHNICAL FIELD

The present invention relates to a chimeric protein containing an intramolecular chaperone (IMC) like sequence linked to a target protein. In particular, the invention relates to a chimeric protein containing an IMC like sequence linked to an insulin precursor.

The present invention also relates to a process for obtaining a correctly folded insulin-precursor-containing chimeric protein, comprising, *inter alia*, contacting an incorrectly folded chimeric protein containing an IMC like sequence linked to an insulin precursor with at least one chaotropic auxiliary agent. The present invention further relates to an assay for screening an amino acid sequence for the ability to improve folding of an insulin precursor using a chimeric protein containing an IMC like sequence linked to an insulin precursor.

1.2. BACKGROUND ART

1.2.1. INTRAMOLECULAR CHAPERONES AND PROTEIN FOLDING

Molecular chaperones are defined as a class of proteins that assist correct folding of other polypeptides but are not components of the functional assembled structure (Shinde and Inouye, *TIBS*, 1993, 18:442-446). Intramolecular chaperones (IMCs) are part of the precursors of the target proteins to be folded and in their absence the target protein molecules do not have enough information for proper self-folding (Inouye, *Enzyme*, 1991, 45:314-321). Unique features of IMCs include: a) the IMC and the target protein are linked by a peptidyl bond forming a single polypeptide; b) the IMC is absolutely required for the formation of active conformation of the target protein, but not required for the function of the target protein; c) upon completion of the protein folding, the IMC is removed either by autoprocesing or by another endopeptidase; d) the IMC does not function as a catalyst, i.e., one IMC molecule is able to refold only one molecule of the target protein; and e) the IMC is a highly specific "tailor-made" polypeptide which works only for the target proteins (Inouye, *Enzyme*, 1991, 45:314-321).

Recently, it has been shown that an IMC or propeptide can help the target protein fold intermolecularly, i.e., the IMC or propeptide is not linked to the target protein via a peptidyl bond, but rather is added to the folding reaction as a separate peptide (U.S. Patent

No. 5,719,021). However, it is noteworthy that the propeptide used in the U.S. Patent No. 5,719,021 is the natural propeptide of the target protein or a propeptide of a polypeptide that has the same function of the target protein and the polypeptide also has an amino acid sequence that is similar to the target protein. In addition, the intermolecular
5 reaction described in the U.S. Patent No. 5,719,021 must be carried out in a buffered ionic aqueous medium favoring hydrophobic interaction.

Examples of IMCs include the propeptides of subtilisin, α -lytic protease, carboxypeptidase Y and ubiquitin (Shinde and Inouye, *TIBS*, 1993, 18:442-446). Certain characteristics of an IMC sequence of subtilisin include: a) the IMC contains a higher
10 percentage of charged amino acid residues than the target protein; b) the distribution of these charged residues within the IMC is extremely uneven, i.e., the N-terminal half contains more positively charged residues than negatively charged residues and the C-terminal half contains more negatively charged residues than positively charged residues; c) Ser and Thr residues within the IMC are also unevenly distributed; d) the IMC contains a
15 reactively high content of aromatic residues; and e) the IMC contains a hydrophobic sequence of 9 residues (Inouye, *Enzyme*, 1991, 45:314-321). A similar bias towards charged residues is also observed in α -lytic protease and carboxypeptidase Y (Inouye, *Enzyme*, 1991, 45:314-321).

20 1.2.2. AMINO ACID SEQUENCE OF MATURE HUMAN GROWTH HORMONE

The amino acid sequence of mature human growth hormone (hGH) is disclosed in Ikehara et al., *Proc. Natl. Acad. Sci. USA*, 1984, 81:5956-5960. There is no suggestion in the art that mature hGH or any portion thereof can function as an IMC or propeptide.

Actually, mature hGH or any portion thereof can not be considered a propeptide at all
25 because by definition, any pre, pro, or prepro sequence is removed from a mature sequence.

1.2.3. HUMAN INSULIN STRUCTURE

Insulin is a well-defined peptide with known amino acid sequence and structural
30 characteristics (Watson et al., *Recombinant DNA--A Short Course*; Scientific American Books, W. H. Freeman Co., New York, 1983, pp. 231-235; Norman and Litwack, *In Hormones*, Academic Press, New York, 1987, pp. 264-317). This hormone consists of two separate peptide chains which are the A chain (21 amino acids) and the B chain (30 amino acids) joined by disulfide bridges as indicated in Figure 1B. Proinsulin is the
35 biological precursor of insulin and is a single peptide chain formed when the A and B chains are connected by the C peptide (Figure 1A).